Nutritional ‘Omics’ Technologies for Elucidating the Role(s) of Bioactive Food Components in Colon Cancer Prevention

New Nutrition, Proteomics, and How Both Can Enhance Studies in Cancer Prevention and Therapy

Helen Kim

Department of Pharmacology and Toxicology, University of Alabama at Birmingham, 1918 University Blvd, Birmingham, AL 35294

ABSTRACT The increased application of MS technologies to nutrition and cancer prevention research has enabled unique insights into the health benefits of polyphenols. Polyphenols are phytochemicals that appear to have chemical properties that provide valuable health benefits when ingested. In particular, experiments have suggested that grape seed proanthocyanidins, oligomers of the catechin family of polyphenols, may have health benefits, possibly due to their capacity to be oxidized. Two-dimensional gel proteomics technology identified specific rat brain proteins that were differentially affected after ingestion of grape seed extract. Beneficial changes in the expression of these proteins were observed relative to changes seen in the brains of Alzheimer disease patients at autopsy and of transgenic mouse models of dementia. These findings were consistent with the hypothesis that grape seed polyphenols may have neuroprotective activity. Previous experiments showed that grape seed extract was significantly chemopreventive in a rat model of breast cancer, but this depended on the specific diet in which the grape seed was administered. Thus, phytochemicals such as polyphenols may have health benefits in mammalian tissues unrelated to classical nutritional deficiency models. This report illustrates how experimental approaches that combine proteomics technologies with a dietary intervention with specific phytochemicals in normal animals can enhance studies on cancer prevention and treatment. J. Nutr. 135: 2715–2718, 2005.

KEY WORDS: • 2D gels • chemoprevention • nutriproteomics • diet and cancer • chemotherapy

The concept that the consumption of foods, or food patterns, can prevent both deficiency diseases and prevent chronic diseases dates back to antiquity. Current nutrition research is using sophisticated technologies to identify the molecular basis for the activity of various dietary chemicals. For example, these techniques may permit the identification of proteins whose expression are affected by such compounds in normal tissues and allow for the identification of proteins that mediate normal cellular functions supported by phytochemicals. As such, these hitherto unidentified proteins may be potential players in the disease process that may develop in the absence of the phytochemicals. In this report we discuss various aspects of two-dimensional (2D) gel proteomics technology that contributed to the identification of specific proteins in rat brain that were affected differentially by dietary administration of grape seed extract (GSE). GSE is a commonly available dietary supplement, to which anti-oxidant properties were first described by Masquelier et al. (1). Previous experiments that suggested that the protective effects of GSE against carcinogen-induced rat mammary cancer might be diet context specific have generated valuable tissues for proteomics analysis. The concept that the physical patterns of protein spots resolved on 2D gels themselves is a tool with which cancer and its prevention or treatment can be studied, irrespective of the identities of the specific proteins within the spots, is presented as an analytical end in itself.

MATERIALS AND METHODS

Generating brains for proteomics analysis of actions of GSE in normal rat brain. Normal weanling female Sprague Dawley rats (Charles River), were fed an AIN-76A diet (Teklad Industries) (2)
The animal experiment that generated brain samples for proteomics analysis. Sprague Dawley female rats (35 d old, n = 5) received either AIN-76A or supplemented with 5% GSE. Animals were euthanized at 6 wk, proteins in the whole-brain homogenates were resolved on replicate 2D gels (shown schematically at the bottom), and analyzed by image and statistical analysis to determine those gel spots that were significantly different between the 2 dietary groups due to the GSE treatment. These spots were excised and processed for MS analysis to identify the polypeptides.
Proteomic signatures of the effect of GSE on rat brain may provide insights into its neuroprotective and chemopreventive effects. In other words, in the molecular pathways the proteins involved represent may provide unique insights into a particular cancer-related phenotype (e.g., angiogenesis, activation of protein tyrosine kinases, etc.) impacted by a specific polyphenol. Ultimately, such information will be the basis for the development of preventives or therapeutic reagents.

While an important goal of this research is to understand the role of polyphenols in carcinogenesis, the pharmaceutical industry will be more interested in identifying effective preventive or therapeutic agents using high throughput assays without necessarily needing to understand the mechanisms or what proteins they affect. Once candidate compounds are identified; however, knowing what are the target proteins or pathways is invaluable in developing compounds with higher specificity or efficacy. Thus, both basic cancer research and targeted chemopreventive or chemotherapeutic compound discovery can ultimately involve searching for “significant” proteins. This search can be greatly enhanced by proteomics approaches that identify changes in patterns of protein expression and modifications in living systems in response to specific stimuli. Aside from the high throughput nature of the technology, proteomics allows rigorous analysis of changes in patterns of protein expression without an a priori prediction of which proteins are involved.

An illustrative example of the application of GSE to breast cancer prevention has been recently demonstrated (7). Based on previous demonstrations in cell culture experiments that grape and tea polyphenols have anticancer properties (9–14), we hypothesized that GSE might inhibit carcinogen-induced breast cancer in rats. Dietary supplementation with 5% GSE inhibited the incidence of DMBA-induced mammary tumors in Sprague-Dawley rats; an unexpected result was that this chemopreventive efficacy of the GSE was seen when it was administered in 4% rodent diet, a diet based on plant proteins (soy, barley, and wheat) vs. milk proteins in AIN-76A (both diets from Teklad Industries). Earlier studies showed that administering GSE to rats did not inhibit breast cancer but did inhibit colon cancer (15); whether the differences between our results and those previously reported were due to dose or dietary differences is not known at present. Our results (7) suggest that the composition of the diet in which a purported chemopreventive compound is administered can determine whether the bioactivity of the compound being tested is expressed.

TABLE 1

Proteomics of actions of GSE in rat brain

<table>
<thead>
<tr>
<th>Protein identity1</th>
<th>Functional category2</th>
<th>MOWSE score3,4</th>
<th>Gene accession number</th>
<th>LC-MS/MS5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat shock protein 60</td>
<td>Chaperone</td>
<td>1.26E-045</td>
<td>P19227</td>
<td>Yes</td>
</tr>
<tr>
<td>Heat shock protein 70</td>
<td>Chaperone</td>
<td>110</td>
<td>gi4103877</td>
<td>Yes</td>
</tr>
<tr>
<td>Heat shock protein 71</td>
<td>Chaperone</td>
<td>105</td>
<td>gi123644</td>
<td>Yes</td>
</tr>
<tr>
<td>Creatine kinase brain beta chain</td>
<td>Energy (generation)</td>
<td>1.66E+05</td>
<td>P07335</td>
<td>Yes</td>
</tr>
<tr>
<td>α and γ enolases</td>
<td>Energy (utilization)</td>
<td>6.6E+05</td>
<td>P04764</td>
<td>Yes</td>
</tr>
<tr>
<td>Glial Fibrillary Acidic Protein</td>
<td>Cytoskeleton</td>
<td>88</td>
<td>P47819</td>
<td>Yes</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Cytoskeleton</td>
<td>93</td>
<td>gi202368</td>
<td>Yes</td>
</tr>
<tr>
<td>Actin</td>
<td>Cytoskeleton</td>
<td>96</td>
<td>gi113307</td>
<td>Yes</td>
</tr>
</tbody>
</table>

3 The molecular weight search engine score generated for each putative identification at MASCOT. Scores above 71–75% are considered valid matches.
4 The scores expressed were generated within PS1 software (Applied Biosystems, Foster City, CA). Scores above E-04 are considered significant.
5 LC-MS/MS was carried out for a number of the identifications generated by peptide mass fingerprint analysis, for confirmation.

FIGURE 2 Representative images of the 2D gels of the rat brain homogenates. These images of 2 real gels show the patterns of protein spots resolved by the orthogonal separation modes, isoelectric focusing (IEF) in the first dimension and SDS-PAGE in the second. The image analysis via PDQuest and subsequent statistical analysis determined that the spots indicated by the circles and rectangles, respectively, differed between the 2 groups of gels in their intensities, and in their variances, respectively.
The molecular basis for the differences in efficacy of the GSE against the DMBA-induced mammary tumors between the 2 diets is not known. Thus, the mammary tissues from the animals in these studies are prime candidates for analysis by 2D gel or other proteomics methods in combination with MS. A simple scenario for analysis is shown in gel or other proteomics methods in combination with MS. A simple scenario for analysis is shown in Figure 3. An altered pattern of protein expression induced by the DMBA carcinogen in mammary tissues can be determined, then compared with how this pattern differs in mammary tissues from animals that began ingesting GSE prior to the DMBA treatment that were subsequently protected against DMBA-induced mammary tumors. It is important to understand that until the very last set of images, the identities of the proteins in the spots do not need to be obtained for the image analysis data to be meaningful.

This report was not meant as a comprehensive review of proteomics, cancer research, or Alzheimer’s disease research. Instead, the goal was to document the health benefits, including chemopreventive activity, of GSE in different rat organs, and to demonstrate how the same proteomics technology that identified and rigorously characterized the proteins affected by GSE in brain could be extended to the study of tissues from cancer studies. Last, but not least, an objective was to emphasize that the “new” nutrition includes diet or dietary components protecting against diseases such as cancers, with effects at a time point distal to the time of ingestion of the dietary component.

LITERATURE CITED